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(54) Title: **DIETARY MODIFICATIONS TO IMPROVE FERTILITY**

(57) Abstract: The invention relates to products and methods for increasing the productivity in farming by improving the fertility of healthy farming mammals. More in particular, the invention relates to products and methods for improving the fertility of animals by reducing placental insufficiency. According to the invention, this is obtained by providing a product which is enriched in L-arginine. The arginine products of the invention may be applied to improve fertility, which may be used in improving the yield in viable offspring of an animal livestock.

Title: Dietary modifications to improve fertility

The invention relates, generally, to products and methods for increasing the productivity in farming by improving the fertility of healthy farming mammals, in particular pigs. More in particular, the invention relates to products and methods for improving the fertility of mammals by
5 improvement of placentation in an animal, in particular by improvement of placentation which results from improved placental angiogenesis.

The invention is applicable to mammals in general, including humans. In the description below, the invention will mainly be illustrated with reference to pigs, but is not limited thereto.

10 Placental insufficiency is the main reason for fetal mortality, stillbirth and early neonatal mortality. This is particularly a problem in farming, where a high production of viable offspring is desired. Healthy European pigs, for example, produce an average of about 12 piglets per litter, while the average loss is approximately 1 piglet per litter due to late fetal
15 mortality, 1 piglet per litter due to stillbirth and 1 piglet per litter due to early neonatal mortality.

The term "fertility" as used in the present application and claims, is to be understood in a broad sense, *viz.* this term not only covers a decreased embryonic and fetal mortality, but also a decrease in perinatal and early
20 neonatal losses of offspring, as well as a decrease in within-litter variation in development at birth. The improved fertility is reflected *e.g.* in an increased production of offspring for farming animals, such as pigs, or in an increased chance for viable offspring in case of animals, such as bovine or equine species or humans, which generally give birth to only a single individual per
25 parturition.

Perinatal mortality is defined as the mortality within a few days around birth, *viz.* all mortality within a day or two before parturition, mortality during and immediately after parturition and all early-neonatal mortality.

In a commercial pig-breeding unit, the overall profitability rises with an increasing number of piglets weaned per sow per year. The latter depends on age at first farrowing, breeding life expectancy, the prolificacy of the sows in the herd (*i.e.* litter size), number of farrowings per sow per year
5 and perinatal and pre-weaning mortality rate. Considering the fact that for each weaned piglet more than two oocytes were initially fertilized, it is evident that piglet mortality during pregnancy, parturition and lactation imposes a substantial loss to the farmer.

In order to prevent fetal mortality and thus improve the fertility of
10 animals, it has *inter alia* been suggested to increase uterine capacity by genetic selection, as described for pigs by Leymaster *et al.* (Proceedings of the 3rd World Congress on Genetics Applied to Livestock Production, XI, (1986) 209-214) or by selecting for increased placental efficiency (Ford, Journal of Reproduction and Fertility, Supplement 52 (1997) 165-176; Wilson *et al.*
15 Journal of Animal Science, 77 (1999) 1654-1658). Although it is possible to select for uterine capacity, this route requires considerable time and effort, and is particularly difficult because uterine capacity is not a simple trait to measure.

The object of the present invention is to provide for methods and
20 products for improving the fertility of animals, which do not have the above-mentioned disadvantages, *viz.* which are, amongst others, relatively simple to apply using existing facilities. It was found that a specific dietary modification, which involves providing in the animals a dosage of arginine, preferably at specific periods during gestation, which dosage is substantially higher than
25 normal dosages, may improve the fertility of the animals by reducing placental insufficiency. The invention is particularly suitable when applied to gestating sows. Normally animal feed contains about 5-15 gram arginine per kg feed. Under normal feeding conditions this amounts to a daily dosage of about 65-200 mg arginine per kg bodyweight (kgbw) of the fed animal. Therefore, in
30 a first aspect, the invention relates to an animal feed suitable for feeding a

gestating animal, which animal feed is enriched in arginine, such that a daily dosage of more than 200 mg arginine per kg bodyweight of said animal is provided upon feeding. Preferably this dosage is more than 400 mg/(kgBW day).

Without wishing to be bound to theory, it is assumed that arginine
5 improves angiogenesis and thus the vascularization of the placenta, by which the development of embryos and fetuses in the uterus is improved. As a result, a better placentation is observed in the animal by which a higher fertility and/or production of more viable offspring is obtained.

The effect of arginine on the animal's fertility in general and
10 especially that of swine, is particularly surprising in view of a report by the National Research Council (U.S.) entitled "Nutrient Requirements of Swine" (National Academy Press (1998, 10th rev. ed.), which is a standard reference for swine breeders. According to this report, swine during pregnancy can synthesize arginine at a rate sufficient to meet most or all of their needs.
15 Accordingly, in the ideal ratio of amino acids for maintenance listed in this report, the value of arginine is negative to reflect the fact that arginine synthesis can satisfy all the maintenance needs. The daily arginine requirement of gestating sows (both on true and apparent ileal digestible basis) is listed as 0 g/day, for sows having a body weight at breeding of 175 kg
20 or more. Furthermore, according to this report, excessive supplements of arginine is even undesirable, since it can reduce feed intake and growth rate.

Wu *et al.* (J. Nutr. 128 (1998) 2395-2402) studied the results of dietary protein deficiencies and noted that arginine concentrations in placenta and endometrium were lower in protein-deficient gilts. Feeding of arginine is,
25 however, not disclosed, nor suggested in this publication.

Furthermore, Wu *et al.* in Curr. Opin. Clin. Nutr. Metab. Care 3 (2000) 59-66, describe that L-arginine administration is beneficial in improving reproductive functions. Although it is suggested in this publication that L-arginine administration may be a means to prevent preterm birth in
30 women, this is attributed to nitric oxide (NO) inhibiting uterine contractility

and maintaining uterine quiescence as a result of the vasodilatation effect of NO, leading to increased blood flow. The effect of arginine on the angiogenesis and thus the vascularization of the placenta, is not disclosed nor suggested. All effects mentioned by Wu *et al.* (2000) in relation to female fertility thus relate
5 to the use of arginine in late gestation.

In the present invention the focus is on the possibility to use arginine in critical periods of placental angiogenesis to stimulate blood vessel formation. Thus, the present invention provides for selective induction of extra angiogenesis for fetuses growing under ischemic conditions, *viz.* in conditions
10 where the supply oxygen and nutrients is limited. As a result, improved fertility, as defined above, is obtained in accordance with the present invention.

Preferably the animal feed of the present invention is enriched in arginine, such that a dosage of 200 - 1300 mg/(kgBW day) arginine is provided
15 upon feeding, more preferably 250 - 1000 mg/(kgBW day), most preferably 250-650 mg/(kgBW day).

The desired concentrations of arginine in the food, required to obtain the suitable dosages of arginine are most conveniently obtained by using a premix. Such a premix may comprise other dietary supplements and may be
20 mixed with conventional animal feed to obtain the animal feed of the invention. A premix in accordance with the present invention may contain typically 1 to 50 wt.% arginine, but this depends mainly on the amount of premix that is to be used to provide the final feed composition. All amounts expressed in weight ratios in the present description and claims are calculated
25 on a dry matter basis. The amount of premix used varies usually between 0.1 to 20% based on the total weight of the mixed feed.

A feed or a premix for a feed according to the present invention is suitably prepared by supplementing arginine, preferably synthetic arginine, to an animal feed (or premix therefore). Such a feed, which is particularly
30 suitable for gestating sows, may contain added arginine in such an amount

that the total arginine/lysine (w/w) content is higher than 1.5, preferably higher than 1.75, preferably higher than 2.0, more preferably higher than 2.25. More preferably, one or more other amino acids are present in the feed in the following ranges (expressed relative to lysine (w/w)).

5

	Preferred	More preferred
Total methionine + cystein	>0.55	0.55-0.70
Threonine	>0.60	0.60-0.80
Tryptophan	>0.15	0.15-0.25

Typically, the amounts (relative to lysine (w/w)) for methionine + cystein, threonine and tryptophan are about 0.60, about 0.70 and about 0.18, respectively. These amounts of amino acids are particularly suitable to provide
10 a feed which covers the requirements as to maintenance, growth and reproduction of gestating sows.

Preferably, the total amount of lysine in the feed for gestating sows is below 0.8 wt.%, more preferably in the range of 0.5 to 0.75 wt %. Although higher amounts of lysine are possible, this is generally not necessary, and thus
15 not preferred for economical reasons.

The arginine, as well as the other amino acids, may be added in the form of the pure amino acid compound (typically synthetically obtained). It is also possible to use polypeptides, including proteins, which are rich in the desired amino acid.

20 Furthermore, the feed according to the present invention, in particular when used as feed for (gestating) sows, preferably comprises calcium. The concentration of calcium is preferably below 1.0 wt%, preferably below 0.8 wt.%.

The present invention is also directed to a method for increasing the
25 breeding productivity of an animal livestock comprising feeding at least one gestating animal of said livestock with 200 - 1300 mg arginine per kg

bodyweight of said animal. Preferably 200 - 1300 mg/(kgBW day) is feeded, more preferably 250 - 650 mg/(kgBW day).

By feeding the animal feed in accordance with the invention, a significant increase in the production of animals is observed. Moreover, the within-litter variation in development at birth (*e.g.* birthweight) decreases significantly, thus contributing to an improved fertility as defined herein-
5 above. For pigs, the improvement obtained according to the invention may be from one up to three piglets per litter, corresponding to an increase in production of 8%, or even more. For other animals, bearing usually only a
10 single offspring at a time, similar results may be obtained, that is to say, a fertility which is significantly improved with respect to the same non-treated animals, as reflected by an improved chance for viable offspring.

Furthermore, it has been found that the method of the invention is very successfully carried out by feeding the animal in one of the so-called
15 critical periods. It was found that normally during gestation, animals such as pigs, go through at least three of such critical phases, which are characterized by a dramatic drop in embryo or fetus count. For pigs, the initial (*i.e.* at day 0 of gestation, directly after fertilization) number of viable embryos is about 17. After about 35-40 days of gestation, this number drops considerably. This
20 period corresponds to the period of embryo implantation and is preceded by a period of rapid placenta growth, which takes place at about day 14-30. In the period which follows the embryo implantation, the fetal count is more or less stable. Then, at day 55-75 again a considerably drop in viable fetuses is observed. This period corresponds to the period wherein the placenta reaches
25 its maximum size. After this period the fetal count is stable again. Finally, at about day 100, which for pigs corresponds to the so-called perinatal period, again a drop in viable fetuses is observed, which is believed to be the result of uterine crowding.

In a preferred embodiment of the method of the invention, the
30 feeding takes place in one or more of the above-mentioned critical periods.

Thus, it is preferred to carry out the feeding during the period of embryo implantation in the gestating animal. It is also preferred to carry out the feeding of the animal in accordance with the present invention during the period of placenta growth, which coincides with embryo implantation.

5 For pigs, this means that the feeding in accordance with the invention is carried out during days 14-30 and days 105-115 of gestation.

Arginine obtained from plant and animal proteins is predominantly present in the L-isomeric form. Although the D-isomer may be used as well, it is not preferred from a practical point of view because of the low commercial
10 availability of D-arginine. The arginine used in accordance with the present invention may be in its pure form, which may be obtained commercially e.g. in the form of crystals. It is also possible, and sometimes preferred for economic considerations, to provide the arginine in the form of proteins or other polypeptides, which are particularly rich in arginine. An example of such an
15 arginine rich product is sesame seed. When using arginine in a proteinaceous or polypeptide form, care must be taken, however, that the amount of nitrogen introduced in the diet is not too high, since this may cause environmental problems.

The method, the animal feed and the premix according to the
20 present invention, may be applied to various animals, in particular mammals. Preferred mammals are those that are agriculturally interesting, especially those selected from bovine species, equine species, porcine species, and ovine species. Also feeding to pet animals is envisaged.

Arginine or a product which is relatively rich in arginine, such as
25 the above-mentioned sesame seeds, may also be used in the preparation of a food supplement for the reduction of placenta insufficiency of an animal. Such a food supplement may be administered to animals, including humans, to improve fertility.

The present invention will be illustrated by the following example.

Example: Oral L-arginine supplementation of sows during pregnancy

Material and methods

The experiment was performed with 197 sows that had already
5 produced at least one litter. Sows in the control group (n=95) received a normal
diet during gestation. Sows in the L-arginine group (n=102) received a normal
diet from insemination until day 13 of pregnancy, an L-arginine supplemented
diet from day 14 until day 31, again the control diet from day 32 until day 104
and again the L-arginine supplemented diet from day 105 until farrowing.

10 The L-arginine supplemented diet was the same as the control diet,
except for the addition of 10 g L-arginine per kg.

Results

The farrowing rate in the control group and L-arginine group was
74% (70/95) and 85% (87/102), respectively.

15 After correction for the effect of parity on litter size, the sows in the
L-arginine group had significantly more piglets per litter than the sows in the
control group (Table 1). The piglets in the L-arginine group had on average a
somewhat lower birth weight (not significant) but this was completely due to
the fact that the litters were larger in this group. After correction for litter
20 size, the birth weight of the piglets was not different between both groups.

Table 1. The effect of L-arginine supplementation during critical periods of prenatal development on litter size and birth weight.

	Control group	L-arginine group
Number of litters	70	87
Total number born + mummies	11.77 \pm 0.33	12.74 \pm 0.31*
Total number born (TNB)	11.75 \pm 0.33	12.69 \pm 0.31*
Number born alive (NBA)	10.92 \pm 0.32	11.77 \pm 0.30+
Birth weight TNB, g	1604 \pm 29	1563 \pm 27
Birth weight TNB corrected for LS, g	1576 \pm 25	1578 \pm 23
Birth weight NBA, g	1627 \pm 29	1584 \pm 27
Birth weight NBA corrected for LS, g	1603 \pm 26	1597 \pm 24

* $p < 0.05$; + $p = 0.055$

5

Stillborn piglets were found in 33 sows of the control group (47.1%) and in 41 sows of the L-arginine group (47.1%). The average number of stillborn piglets in litters with stillborn piglets was 1.91 ± 0.25 and 1.86 ± 0.22 , respectively (not significantly different).

10

From these results, it follows that oral arginine supplementation of sows during critical periods during pregnancy has a positive effect on farrowing rate.

15

Furthermore, arginine supplementation of sows during critical periods during pregnancy has a significant effect on litter size, both total number born and number born alive (± 1 piglet extra).

Also, oral arginine supplementation of sows during critical periods during pregnancy has no effect on the percentage of litters with stillborn piglets and no effect on the number of stillborn piglets in litters with stillborn piglets.

20

Claims

1. Animal feed suitable for feeding a gestating animal, which animal feed is enriched in arginine, such that a daily dosage of at least 200 mg arginine per kg bodyweight of said animal (kgBW) is provided upon feeding.
2. Sow feed according to claim 1, which feed is enriched in arginine,
5 such that a dosage of 200 - 1300 mg/(kgBW day) arginine is provided upon feeding, preferably 250- 650 mg/(kgBW day).
3. Sow feed according to claim 1 or 2, which contains 1.25 to 10 wt% arginine, preferably 1.5 to 10 wt. %
4. Sow feed according to any of the previous claims, comprising added
10 arginine in such an amount that the total arginine/lysine (w/w) content is higher than 1.5, preferably higher than 1.75, more preferably higher than 2.0, most preferably higher than 2.25.
5. Feed or premix, in particular for feeding sows, according to any of the previous claims, comprising one or more amino acids in an amount relative
15 to lysine (w/w) in the following ranges:
Methionine + cystein: > 0.55;
Threonine: > 0.60;
Tryptophan: > 0.15.
6. Sow feed or premix according to claim 5, comprising one or more
20 amino acids in an amount relative to lysine (w/w) in the following ranges:
Methionine + cystein: 0.55-0.70;
Threonine: 0.60-0.80;
Tryptophan: 0.15-0.25.
7. Feed according to any of the previous claims, wherein the total
25 amount of lysine in the feed is below 0.8 wt. %.
8. Feed according to any of the previous claims, wherein the amount of Ca^{2+} is below 1.0 wt%.

9. Feed or premix according to any of the previous claims, wherein said arginine is of synthetic origin and/or in the form arginine rich polypeptide, preferably arginine rich protein.
10. Premix containing arginine in a sufficient amount to obtain the
5 animal feed according to any of the previous claims upon mixing.
11. Method for increasing the breeding productivity of an animal livestock, in particular a livestock of porcine species, comprising feeding at least one gestating animal of said livestock with a daily dosage of 200 - 1300 mg arginine per kg bodyweight of said animal.
- 10 12. Method according to claim 11, which comprises feeding a feed according to any of claims 1-9, which feed is optionally obtained by mixing a premix according to claim 10.
13. Method according to claim 11 or 12 , wherein said feeding takes place during critical periods for placental angiogenesis.
- 15 14. Method according to claim 11-13, wherein said feeding takes place during periods of placental angiogenesis and growth.
15. Method according to any of the previous claims 11-14, wherein said feeding takes place during the perinatal period of gestation.
16. Method according to any of the previous claims 11-15, wherein said
20 animal is a pig and wherein said feeding takes place at days 14-30 and at days 105-115 of gestation.
17. Method, animal feed or premix according to any of the previous claims, wherein said animal is a mammal selected from bovine species, porcine species, or ovine species.
- 25 18. Use of arginine or an arginine enriched product for increasing the breeding productivity of an animal livestock.
19. Use of arginine or an arginine enriched product in the preparation of a food supplement for the improvement of placentation in an animal.
20. Use according to claim 19, wherein said improvement of
30 placentation results from improved placental angiogenesis.